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Targeted precursor addition to increase baked flavour in a low acrylamide potato-based matrix

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Abstract

The aim of this study was to increase the baked flavour of low acrylamide potato products. Strecker aldehydes and pyrazines make an important contribution to the flavour of potato products and are formed alongside acrylamide in the Maillard reaction. However, the Maillard reaction can be directed in favour of aroma formation by selecting appropriate precursors and intermediates based on the fundamental chemistry involved.

Selected precursors were added to potato dough prior to baking. Addition of glycine and alanine together doubled high impact pyrazines and addition of 2,3-pentanedione or 3,4-hexanedione also promoted the formation of key trisubstituted pyrazines. Quantitative descriptive profiling of sensory attributes indicated that baked flavour was increased most when both Strecker aldehydes and pyrazines were increased together. This work shows that it is possible to enhance baked flavour in low acrylamide products by adding a specifically targeted combination of amino acids and key intermediates, without increasing acrylamide concentration.

Keywords: potato, complex matrix, acrylamide mitigation, flavour, Maillard, pyrazines

1. Introduction

Fundamental research on exploring mitigation approaches for process contaminants, such as acrylamide, is an important area for the food & beverage industries. Specifically, greater understanding is required on maintaining the flavour quality of products as mitigation may alter flavour profiles. Model systems are useful approaches to study specific pathways, however this approach should also be supplemented with work on complex food systems to allow better translation into real food products. The use of simplified real food matrices is seen as a bridge between simple model systems and complex real food systems, and more studies using this approach can help in uncovering interaction and synergistic effects which may not occur in simple model systems.

High temperature thermal processing of asparagine rich foods results in the formation of acrylamide during the Maillard reaction (Mottram, Wedzicha, & Dodson, 2002; Stadler, Blank, Varga, Robert, Hau, Guy, et al., 2002) and it is classified as a Class 2a probable carcinogen in humans (International Agency for Research on Cancer, 1994). As flavour and browning also develop during this process, it is often a challenge to mitigate acrylamide formation from the viewpoint of food quality. Several strategies have been developed for this purpose however only a few of them mitigate acrylamide formation whilst allowing the cooked flavour and colour to develop. Selection of low sugar potato varieties and the use of asparaginase in bakery products have been proposed as the best mitigation strategies (Palermo, Gökmen, De Meulenaer, Ciesarová, Zhang, Pedreschi, et al., 2016).

Lowering thermal input is a convenient approach for baked foods for the mitigation of acrylamide formation (Gokmen, Acar, Koksel, & Acar, 2007; Taeymans, Wood, Ashby, Blank, Studer, Stadler, et al., 2004), but as this strategy depends on limiting the overall Maillard reaction, the final food product lacks baked flavour.

A pool of reactive intermediates is formed from the reaction between sugars and amino acids in the intermediate stage of the Maillard reaction (Yaylayan, 1997). The composition of this pool determines the flavour and browning characteristics of the final product (Yaylayan, 2003) and also influences acrylamide formation. The baked flavour is largely obtained by Strecker aldehydes and trisubstituted alkylpyrazines which are formed from the intermediates found in the pool. Addition of amino acids and other precursors can be used to alter the composition of the pool to manipulate flavour formation.

Strecker aldehydes are formed from free amino acids during the Maillard reaction via a number of different pathways (Yaylayan, 2003). In particular, α-dicarbonyl compounds transform amino acids to their Strecker aldehydes via decarboxylation. Strecker aldehydes which are important for aroma are 2-methylpropanal (malty), 2-methylbutanal (malty, cacao like), 3-methylbutanal (malty, cacao like), methional (boiled potato) and phenylacetaldehyde (floral, honey like) and they are formed from valine, isoleucine, leucine, methionine and phenylalanine, respectively (Martin & Ames, 2001). In addition, the Strecker aldehydes formaldehyde and acetaldehyde, formed from glycine and alanine respectively, contribute to the pool of intermediates and can be precursors of methyl and ethyl substituted pyrazines, respectively (Amrani-Hemaimi, Cerny, & Fay, 1995; Low, Koutsidis, Parker, Elmore, Dodson, & Mottram, 2006).

α-Dicarbonyl compounds are converted into α-amino carbonyl compounds during the Strecker degradation of amino acids, whereas the reaction of α-hydroxycarbonyl compounds and ammonia may produce α-aminocarbonyl compounds via Amadori rearrangement (Yaylayan, 2003). N-terminal amino acids in peptides have also been shown to transform α-dicarbonyl compounds into their α-amino carbonyl derivatives with a different reaction mechanism to Strecker degradation (Van Lancker, Adams, & De Kimpe, 2010, 2012). Condensation of two α-amino carbonyl compounds produces a dihydropyrazine and subsequent oxidation yields a pyrazine (Shibamoto & Bernhard,

1977). Methyl substitution in pyrazines may originate from methylglyoxal (via 2-aminopropanal or 1-amino-2-propanone), 1-hydroxy-2-propanone (via 2-aminopropanal), 2,3-butanedione and 3-hydroxy-2-butanone (via 3-amino-2-butanone) followed by oxidation of the corresponding dihydropyrazines (Chu & Yaylayan, 2009). Ethyl substitution may come from 2-oxobutanal, 2,3-pentanedione and 3,4-hexanedione via this oxidative pathway (Chu & Yaylayan, 2009). In addition to this oxidative pathway, nucleophilic attack by a dihydropyrazine at the carbonyl centre of formaldehyde or acetaldehyde, followed by the elimination of a water molecule, also yields alkyl substituted pyrazines in a non-oxidative way (Amrani-Hemaimi, Cerny, & Fay, 1995; Cerny & Grosch, 1994).

The pyrazines which we are targeting in this work are the trisubstituted alkylpyrazines with relatively low odour thresholds and baked aroma (2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine) since they are known to contribute to the baked flavour in potato products (Buttery, Seifert, Guadagni, & Ling, 1971; R. Wagner, Czerny, Bielohradsky, & Grosch, 1999; R. K. Wagner & Grosch, 1998). On the basis of the chemistry underpinning the formation of these baked flavour compounds, we propose that with addition of a carefully targeted selection of precursors to a potato-based matrix, we will be able to increase the formation of key aroma compounds whilst maintaining low levels of acrylamide. Glycine and alanine were selected to increase overall Maillard reaction and also as alkylpyrazine precursors. Leucine, isoleucine, valine, phenylalanine, and methionine were selected as Strecker aldehyde precursors. 2,3-Pentanedione (PD) and 3,4-hexanedione (HD) were used as intermediates in alkylpyrazine formation. Moreover, the addition of whey protein hydrolysate was assessed, also together with 2,3-pentanedione and 3,4-hexanedione, as oligopeptides have previously been shown to be very effective in alkylpyrazine formation (Scalone, Lamichhane, Cucu, De Kimpe, De Meulenaer, 2019).

2. Materials and Methods

2.1 Chemicals and Consumables

$^{13}C_3$-Acrylamide was purchased from Cambridge Isotope Laboratories (QMx, Thaxted, UK). Acetonitrile (LC-MS grade), NaCl (reagent grade) and formic acid (LC-MS grade) were purchased from Fisher Scientific (Loughborough, UK). Deionised water was supplied from a MilliQ system at 18.2 MΩ.cm resistivity. Food grade amino acids were supplied from S.A. Ajinomoto OmniChem N.V. (Wetteren, Belgium). Amino acids reference mix solution (2.5 mM each), 2,3-pentanedione (food grade) and 3,4-hexanedione (food grade) were purchased from Sigma-Aldrich (Poole, UK).

2.2 Preparation of Potato-Based Matrix

A potato-based dough was prepared by mixing 342 g potato flake, 90 g waxy maize starch, 4.5 g lecithin, 4.5 g NaCl, 9 g sucrose and 300 g tap water at room temperature. Sucrose and NaCl were dissolved in water for the control formula. Amino acids, 2,3-pentanedione and 3,4-hexanedione were also dissolved in water to incorporate them homogenously into the dough. The amount of added amino acids was four times that of the naturally occurring levels in the potato flake (Table S1, see supplementary material) for all amino acids except Gly. Gly was added at 20- or 100-fold the naturally occurring level in the potato flake. Additionally, the 'all amino acids' recipe contained twice that of the naturally occurring concentrations of each amino acids except asparagine and γ-aminobutyric acid which were omitted from the mix. 2,3-Pentanedione and 3,4-hexanedione were added at 100 mg/kg on a dry basis. Whey protein hydrolysate was added at 5% on a dry basis. Dry ingredients were mixed on setting 1 (Kenwood Mixer) for 30 s and then the aqueous mixture was gradually added during mixing for 1 min. The dough was sheeted by using a pastry sheeter (Rondo, Burgdorf, Switzerland) to a thickness of 1.2 $\pm$ 0.06 mm. The dough was cut into disks of 5 cm and the pieces were pinned. Twenty-four pieces were transferred onto an open mesh baking mat and were baked at 160 °C for 6 min followed by drying at 130 °C for 10 min under full convection mode.

They were cooled down for 5 min on an aluminium foil at room temperature and immediately packed in foil pouches by heat sealing and kept at -20 °C until chemical or sensory analysis.

2.3 Amino Acids Analysis

Ground potato flake (1.0±0.05 g) was extracted with water in three stages using 10, 5 and 5 mL respectively. At each stage, the tube was vortexed for 5 min followed by centrifugation at 6654×g for 5 min. Supernatants were combined in a tube, and 0.2 mL of combined extract was mixed with 0.8 mL acetonitrile. The mixture was centrifuged and a part of the supernatant was filtered through 0.2 µm PTFE filter (Fisher Scientific, UK) into an autosampler vial. External calibration curves of amino acids were built between 1-50 µM in water:acetonitrile mixture (2:8, v:v).

Amino acids were analysed using an Agilent 1200 high-performance liquid chromatography (HPLC) system coupled to a 6410 triple quadrupole mass spectrometer with electrospray ion source (ESI) in positive mode. Chromatographic separation was carried out on a Synchronis HILIC column (150 × 4.6 mm i.d., 3 µm) with a Synchronis HILIC precolumn (10 × 4.6 mm i.d., 3 µm, ThermoFisher Scientific, Waltham, MA, USA). The mobile phase A was 5 mM ammonium formate with 0.5% formic acid in water, and B was 5 mM ammonium formate with 0.5% formic acid in acetonitrile:water (9:1, v:v) at a flow rate of 1 mL/min at 20 °C with gradient elution. Mobile phase A was increased from 10% to 40% for 8 min and then decreased to 10% in 1 min and kept at 10% for 4 min. The total run time was 13 min. The injection volume was 5 µL. ESI source had the following settings: gas temperature 330 °C, gas flow 13 L/min, nebuliser pressure 40 psi, capillary 4000 V. Amino acids were identified by multiple reaction monitoring (MRM) using the ion transitions and parameters previously reported (Kocadağlı, Özdemir and Gökmen, 2013).

2.4 Acrylamide Analysis

157 The ground sample (1±0.05 g) was put into 50 mL centrifuge tube and 2 mL 1 mg/L $^{13}C_3$-acrylamide
158 was added followed by 38 mL water. After vortexing for 20 min, the tubes were centrifuged at
159 8422×g for 15 min at 15 °C. An aliquot of the supernatant was filtered through a 0.2 µm nylon
160 syringe filter (Fisher Scientific, Loughborough, UK) into an autosampler vial. An external calibration
161 curve of acrylamide was built between 0.5-20 µg/L, each containing 50 µg/L $^{13}C_3$-acrylamide.

162 Samples were analysed using an Agilent 1200 high-performance liquid chromatography (HPLC)
163 system coupled to a 6410 triple quadrupole mass spectrometer with electrospray ion source (ESI)
164 in positive mode. Chromatographic separation was carried out using a Hypercarb column (100 × 3.0
165 mm i.d., 5 µm) with a Hypercarb precolumn (10 × 3.0 mm i.d., 5 µm, ThermoFisher Scientific,
166 Waltham, MA, USA). The mobile phase was 0.1% aqueous formic acid at a flow rate of 0.3 mL/min
167 at 30 °C. The injection volume was 5 µL. ESI source had the following settings: gas temperature 350
168 °C, gas flow 10 L/min, nebuliser pressure 35 psi, capillary 3000 V and fragmentor voltage 40 V. The
169 MRM transitions of m/z 72 → 55 (N_2 collision energy (CE), 8 eV) and 72 → 27 (CE, 16 eV) were
170 measured for acrylamide, and the MRM transition of m/z 75 → 58 (CE, 8 eV) was measured for $^{13}C_3$-
171 acrylamide. A dwell time of 100 ms was set for all three MRM transitions.

172 ***2.5 Aroma Analysis***

173 Volatile compounds were collected onto Tenax TA using dynamic headspace extraction. The cooked
174 potato-based matrix was ground and 10 g was transferred into a 250 mL conical flask with a screw-
175 thread neck and 15 mL saturated NaCl solution (35 g/100 mL) was added. The flask was kept at 50
176 °C in a water bath during the extraction. Oxygen-free nitrogen was passed over the sample for 1 h
177 at a rate of 40 mL/min, carrying volatiles onto a preconditioned glass trap (105 mm × 3 mm i.d.)
178 containing Tenax TA (85 mg) (SupelCo, Poole, UK). At the end of the collection, 130.6 ng of 1,2-
179 dichlorobenzene in 1µL methanol was injected onto the trap as an internal standard. Then, the trap

was flushed with nitrogen at a flow rate of 100 mL/min for 10 min to remove water. Volatiles collected on the trap were desorbed using a Turbomatrix ATD system (Perkin Elmer, Beaconsfield, UK) coupled to an Agilent GC-MS system (7890A-5975C). The Tenax traps were desorbed at 300 °C (heating rate 40 °C/s) and cryofocused in a cold trap at −30 °C. Chromatographic separation was achieved on a Phenomenex ZB-Wax column (30 m × 250 µm i.d.× 1 µm). The time-temperature program employed was 10 min at 30 °C, a ramp of 4 °C/min to 250 °C, and hold at 250 °C for 10 min. The carrier gas was He at 1 mL/min. The mass spectrometer was operated in electron ionization mode with an ionizing voltage of 70 eV and source temperature of 200 °C. A scan range of *m/z* 29-450 was employed with a scan time of 0.7 s. The data were controlled and stored using ChemStation.

A series of *n*-alkane standards (C_5-C_{25}) in diethyl ether was run to obtain linear retention index (LRI) values. The identity of the compounds was confirmed by comparing their mass spectra with NIST Mass Spectral Database, authentic compounds and LRI values (Table S2 & S3, see supplementary material). The compounds were semi-quantified by comparing the peak areas of extracted ion chromatograms with that of the internal standard 1,2-dichlorobenzene, using a response factor of 1.

2.6 Sensory Analysis

A trained in-house panel of 11 assessors (10 female, 1 male) were used to estimate sensory changes quantitatively. Samples were presented to assessors with random codes to discuss as a group, with the help of a panel leader, and to develop a consensus vocabulary on the sensory attributes of the cooked potato-based products. References (real food or aroma compounds) were available for the panel to help define the product attributes during four sessions of 1 h (Table S4, see supplementary material). During development of the sensory attributes, panellists were first asked to sniff and then bite into the sample to define aroma, taste, flavour and aftertaste attributes. The day before the

quantitative sensory assessment took place, the panellists were exposed again to the references and samples to standardise the vocabulary associated with defined attributes (30 min). Panellists were asked to score the attributes of the control sample during the training session to obtain average scores. The quantitative sensory assessments were carried out in well-ventilated individual sensory booths controlled at 21±1 °C, in duplicate (replicates were presented on consecutive days). The panellists were given the average scores together with the control sample (introduced with a code 'reference') during the quantitative sensory assessments. Panellists were asked to score each attribute in reference to the control sample on an unstructured 15 cm line, scaled 0-100, with an anchor defining the average score for the reference product, using Compusense @Hand (Compusense Inc., Guelph, Ontario, Canada). The samples, with three-digit random codes, were introduced in a randomised and balanced order, always with a blind control sample in each session. Between samples, panellists were asked to cleanse their palate with warm water.

2.7 Statistical Analysis

One-way analysis of variance was performed on the instrumental data to indicate statistical differences between recipes using the Duncan test. ANOVA and Fisher's LSD multiple comparison test at p = 0.05 were carried out using the statistical programme Senpaq 5.01 (Qi Statistics Ltd., Reading, UK).

3. Results and Discussion

3.1 Concentration of amino acids and reducing sugars in the potato flake

The potato flake contained relatively low amounts of reducing sugars (6.7±0.24 g/kg glucose and 3.7±0.04 g/kg fructose). The concentration of Asn was 2552 mg/kg, similar to previously reported data (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005). Concentrations of free amino acids

225 in the potato flake were determined (Table S1, see supplementary material) and these values were
226 used to increase their initial concentrations 2 to 100-fold by adding amino acids to the recipe. The
227 amount of added Ala, Leu, Ile, Val and Met was 4 times the naturally occurring amount to make
228 their concentrations 5 times that of the control recipe. However, glycine was one of the lowest
229 amino acids in the potato flake and therefore its concentration was further increased to 100-fold to
230 maintain a level similar to alanine. This corresponded to Gly addition of 0.18% and Ala addition of
231 0.21% (4 times the naturally found level) on potato flake basis. An additional recipe was formed by
232 adding all amino acids (except Asn and γ-aminobutyric acid) at twice the naturally occurring levels.

233 ***3.2 Determination of low thermal input conditions to limit acrylamide formation and monitor***
234 ***alkylpyrazine formation***

235 Apart from the time-temperature conditions used in the main body of this paper (baking at 160 °C
236 for 6 min followed by drying at 130 °C for 10 min), two other conditions, one lower and one higher,
237 were also performed during preliminary trials. At lower temperature conditions (baking at 140 °C
238 for 8 min followed by drying at 110 °C for 15 min), either adding amino acids individually or as binary
239 mixtures did not have a significant effect on alkylpyrazine formation (Figure S1, see supplementary
240 material). Only trimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine and 2-ethyl-
241 3,6-dimethylpyrazine were found to be slightly higher when Gly and Ala were added together.
242 Overall, these conditions generated fewer pyrazines than the more severe time-temperature
243 conditions and 2-ethyl-3,5-dimethylpyrazine, one of the key odour-active aroma compounds of
244 interest, was not detected under these conditions at all. The concentration of acrylamide was also
245 very low (10 to 15 ng/g, data not shown). Under these low temperature conditions, the formation
246 of the dicarbonyl/hydroxycarbonyl precursors required for pyrazine formation and acrylamide
247 formation is slow and there were insufficient intermediates formed to significantly increase the
248 concentration of alkylpyrazines. However, the 4-fold addition of Val, Ile, Leu and Met significantly

increased the corresponding Strecker aldehydes, by about 4-fold (Figure S1, see supplementary material). It has been previously shown that Strecker aldehydes are kinetically formed earlier than alkylpyrazines in a potato matrix (Low, Mottram, & Elmore, 2006). This may partly explain increases in Strecker aldehydes but no significant changes in alkylpyrazines.

At a higher temperature (baking at 160 °C for 6 min followed by drying at 140 °C for 8 min) the concentration of acrylamide in the product was 233±43 ng/g (data not shown), compared to 91±8.2 ng/g using the baking conditions discussed in the main body of this article (baking at 160 °C for 6 min followed by drying at 130 °C for 10 min). It was possible to observe the effect of amino acid additions on alkylpyrazine formation under both sets of conditions, but only the results (chemical and sensory) for the moderate thermal input conditions are presented below as the focus of the work was on a low acrylamide products.

3.3 Strecker aldehyde formation and effect of the addition of corresponding amino acids

By adding a targeted mixture of corresponding amino acids (recipe: 4 × Met+Ile+Leu+Val), Strecker aldehydes methional, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal all increased in similar proportions to the added amounts, namely 499%, 440%, 419% and 437%, respectively (Figure 1). Similarly, addition of Phe at twice the natural level (recipe: all amino acids ×2) increased phenylacetaldehyde to 204%. When the targeted Strecker aldehyde precursor amino acids (×4) were added together with Gly (×100) and Ala (×4), the increases were only 370%, 343%, 318% and 326% for methional, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal , respectively. In accordance with this smaller increase, phenylacetaldehyde decreased 31% in 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipe however it was statistically insignificant ($p>0.05$). Similar decreases were also observed in Gly and Ala added samples with respect to control however they were also not significant. This smaller increase in the presence of Gly and Ala is likely to be due to

the fact that amino acids compete with each other for the available dicarbonyls, increasing the Strecker aldehydes derived from Gly and Ala (formaldehyde and acetaldehyde) whilst decreasing the others. This observation was also evident in a previous study in which 1.3% Gly on dry basis was added to a potato model system cooked at 180 °C (Low et el, 2006).

In addition to changes in Strecker aldehydes, dimethyl disulfide and dimethyl trisulfide, the degradation products of methionine and methional, increased 98% and 41% in the 'Met+Ile+Leu+Val' recipe, 139% and 148% in 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipe and 45% and 43% in 'all amino acids' recipe, respectively (Table S2, see supplementary material). These sulfide compounds have very low odour thresholds and they also contribute to the cooked flavour in potato-based matrices (Martin & Ames, 2001).

3.4 Effect of amino acid addition on alkylpyrazine formation

Addition of individual amino acids Gly, Ala, Gln and also Ser+Thr was performed at the higher thermal input condition mentioned above, and there were no changes in alkylpyrazines (Figure S2, see supplementary material). A binary mixture of Ser and Thr without carbonyls has been shown to produce certain alkylpyrazines under pyrolytic conditions (Shu, 1999), and Gln, Ser and Thr have been shown to produce various alkylpyrazines in the presence of reducing sugar at pH 8 and 160 °C (Chen & Ho, 1999). However, these conditions do not correspond to the low thermal input conditions used here. On the other hand, when Gly and Ala were added together significant increases in certain alkylpyrazines were observed at both thermal input conditions (Figure 1; and Figure S2, see supplementary material).

Pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine and 2-ethyl-5-methylpyrazine did not change considerably with amino acid addition with respect to control (Table S2, see supplementary material). As shown in Figure 1,

295 trimethylpyrazine increased by 81% and 134% when Gly was increased 100 fold in the recipes
296 Gly(×100)+Ala and Gly(×100)+Ala+Met+Ile+Leu+Val, respectively ($p<0.05$). In Gly(×20)+Ala recipe,
297 the increase in trimethylpyrazine was 44% without statistical significance ($p>0.05$). This is consistent
298 with the non-oxidative route for formation of pyrazines, where one of the methyl substituents
299 comes from Gly, probably via formaldehyde.

300 Remarkable increases were observed for 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,6-
301 diethylpyrazine, 2,3-diethylpyrazine, and more importantly for the lowest odour threshold
302 trisubstituted pyrazines 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine, when Gly
303 and Ala were added together. The amounts were more than twice or three times that of the control
304 recipe in most cases with statistical significance. The increase in 2-ethyl-3,6-dimethylpyrazine was
305 261%, 210% and 348% in 'Gly(×20)+Ala', 'Gly(×100)+Ala' and 'Gly(×100)+Ala+Met+Ile+Leu+Val'
306 recipes, respectively ($p<0.05$). 2-Ethyl-3,5-dimethylpyrazine increased 98% ($p>0.05$), 142% and
307 155% ($p<0.05$) in 'Gly(×20)+Ala', 'Gly(×100)+Ala' and 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipes,
308 respectively. These trisubstituted pyrazine isomers 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-
309 dimethylpyrazine have odour thresholds (3.6 ng/L air and 0.011 ng/L air respectively), particularly
310 compared to other pyrazines (Table S2) and have been shown to contribute to the baked flavour in
311 a potato matrix (Wagner & Grosch, 1998).

312 Addition of all free amino acids (except Asn and GABA) at twice that of the natural level did not have
313 a significant effect on the alkylpyrazine formation, neither did addition of Gly and Ala separately
314 (see above). However, Figure 1 shows that addition of Gly and Ala together increased the formation
315 of key alkylpyrazines with low odour thresholds in low acrylamide cooked potato-based matrices,
316 whereas the addition of Gly alone did not have this effect, at least in the high temperature baked
317 products. However, Low et al (2006) showed an increase (>100%) in a wider range of pyrazines
318 (particularly 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and

tetramethylpyrazine) when Gly (1.3% on dry basis) was added to mitigate the formation of acrylamide in potato cakes baked at 180 °C. The reason for not observing the effect of individual addition of Gly on alkylpyrazine formation in this study might be due to the low thermal input condition used to prepare low acrylamide potato products, and also the added Gly was 7.2-fold lower.

Both Gly and Ala have many possible roles in the Maillard reaction. They participate in the first step of the Maillard reaction and facilitate the formation of α-dicarbonyl compounds from reducing sugars which are important precursors of pyrazines. However, they can also compete with the other amino acids for Strecker degradation of the available carbonyls. Additionally, Strecker degradation of Gly and Ala yield their Strecker aldehydes (formaldehyde and acetaldehyde respectively) and α-amino carbonyl compounds, both of which are intermediates in alkylpyrazine formation. Formaldehyde and acetaldehyde formed from Gly and Ala also participate in the chain elongation process of sugar-derived α-dicarbonyl compounds and these α-dicarbonyl compounds with ethyl and/or methyl substituents from amino acids may be involved in alkylpyrazine formation through oxidative or non-oxidative pathways. In the oxidative pathway, the dihydroalkylpyrazine intermediates readily oxidise to form the corresponding alkylpyrazines and this has been shown to be a major pathway (Chu & Yaylayan, 2009). Formaldehyde and acetaldehyde formed from Gly and Ala participate in the non-oxidative pathway of pyrazine formation and yield ethyl or methyl substituted alkylpyrazines upon dehydration of the alkylated site of dihydropyrazines.

It is interesting that the 2-ethyl-3,6-dimethylpyrazine is influenced more than 2-ethyl-3,5-dimethylpyrazine by the addition of alanine, suggesting greater incorporation of the alanine backbone into the molecule via the non-oxidative mechanism. The 2-ethyl-3,6-dimethylpyrazine is also formed in greater quantities than the 2-ethyl-3,5-dimethylpyrazine, which was not detected at all under the lower temperature conditions. The 2-ethyl-3,6-dimethylpyrazine can be formed by the

condensation of two molecules of 1-amino-2-propanone (from methylglyoxal, and sterically favoured over 2-aminopropanal) and incorporation of acetaldehyde (from alanine) (Figure S3). The former is readily formed and more reactive than higher molecular weight dicarbonyls (Hofmann, 1999) such as 2,3-pentanedione which is required for the oxidative pathway. This may explain both the greater quantity formed and the greater response to alanine addition. Although it is hard to clarify the mechanism, it is clear that Gly and Ala are effective together in increasing certain pyrazines, especially the lower threshold trisubstituted isomers, in low acrylamide cooked potato model systems.

3.5 Targeted addition of carbonyl compounds as carbon backbones for alkylpyrazine formation

The carbonyl pool formed in the intermediate stage of Maillard reaction can also be manipulated by adding 2,3-pentanedione (PD) and 3,4-hexanedione (HD) to provide the required carbon backbone for alkylpyrazine formation. They can be converted into their corresponding α-aminocarbonyl derivatives which may then participate in the alkylpyrazine formation in the presence of amino compounds. Addition of 100 mg/kg 2,3-pentanedione on a dry basis increased its headspace concentration 15 and 10 times in cooked 2,3-PD and 2,3-PD-Leu-Ile-Met-Val+Phe recipes, respectively. There was no 3,4-hexanedione in the headspace of the control sample and its addition level was 100 mg/kg dry basis as well.

Addition of 2,3-pentanedione alone increased 2-ethyl-3-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine 155% and 121% with respect to control recipe (Figure 2) ($p<0.05$). On the other hand, 2-ethyl-3,6-dimethylpyrazine did not change when 2,3-pentanedione added alone. We speculate that this is because the methyl-substituted second carbon in 2,3-pentanedione is less sterically hindered than the ethyl substituted third carbon and it is preferably transformed into 2-amino-3-pentanone during Strecker degradation. 2-Amino-3-pentanone condenses with 1-amino-

2-propanone, which for the same reason is preferably formed from methylglyoxal instead of 2-aminopropanal, and produces 2-ethyl-3,5-dimethylpyrazine (See supplementary material for the proposed mechanism, Figure S3). Therefore, the addition of 2,3-pentanedione targets the formation of lower odour threshold trisubstituted isomer 2-ethyl-3,5-dimethylpyrazine in the potato matrix. Adding Leu, Ile, Met, Val and Phe together with 2,3-pentanedione increased 2-ethyl-3-methylpyrazine, 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine even more (272%, 146% and 390%, respectively), as more amino acids aided the transformation of α-dicarbonyls into their α-aminocarbonyl derivatives during the Strecker degradation ($p<0.05$). Scalone et al (2019) also suggested this pathway of 2-ethyl-3,5-dimethylpyrazine formation from 2,3-pentanedione in whey protein hydrolysate-glucose model systems (pH 7.8 phosphate buffer) heated at 180 C for 90 min.

Addition of 3,4-hexanedione alone increased 2,3-diethylpyrazine by 84% however, it was not statistically different to the control recipe (Figure 2). A further increase in 2,3-diethylpyrazine (338%) was achieved when Leu, Ile, Met, Val and Phe were also added ($p<0.05$). 2,3-Diethyl-5-methylpyrazine was not detected in the control sample in this set of baking. By the addition of 3,4-hexanedione alone and together with Leu, Ile, Met, Val and Phe, 2,3-diethyl-5-methylpyrazine was detected at levels comparable to 2-ethyl-3,5-dimethylpyrazine. This pyrazine has a roast, potato aroma and a low threshold, and is known to be important in baked potato aroma.

3.6 Effect of whey protein hydrolysate addition on alkylpyrazine formation

Addition of whey protein hydrolysate to enhance the Maillard reaction and also as an amine source when it is added together with 2,3-pentanedione and 3,4-hexanedione was assessed for its effect on alkylpyrazine formation. The total concentration of free amino acids in whey protein hydrolysate was 75 mg/kg and they did not contribute to the free amino acid pool in potato flake, which was

1600-fold higher. Therefore, there were no changes for Strecker aldehydes (Table S3, see supplementary material). On the other hand, all alkylpyrazines increased considerably by the addition of 5% whey protein hydrolysate on dry basis (Figure 3). The differences for some alkylpyrazines were statistically insignificant due to the higher baking variance between replicates in this set of baking although the concentrations were more than doubled with respect to control. Interestingly, a minor increase (30%) was observed only for unsubstituted pyrazine while others increased at least one-fold or two (Table S2, see supplementary material). Pyrazine is formed from two molecules of glyoxal, and vinylic pyrazines were also increased by the addition of whey protein hydrolysate. These changes indicated that whey protein hydrolysate increases overall Maillard reaction and thus aiding alkylpyrazine formation by enhancing the carbonyl pool at the intermediate stage. The pH of the dough was 5.92±0.01 and it did not change by the addition of 5% whey protein hydrolysate (5.92±0.01). It has been shown that the N-terminal amino groups found in peptides are also effective for the formation of pyrazines with a different mechanism that is observed in the Strecker degradation of free amino acids (Van Lancker et al, 2010, 2012). Scaleno et al (2015) showed that whey protein hydrolysate contributes to the formation of pyrazines significantly more than free amino acids during heating whey protein hydrolysate and glucose mixture with phosphate buffer at ph 7.8. Moreover, by addition of 2,3-pentanedione and 3,4-hexanedione together with whey protein hydrolysate, considerably higher amounts of corresponding alkylpyrazines were observed (Figure 3).

3.7 Changes in acrylamide formation by recipe modifications

The concentration of acrylamide in the control recipe was 91±8.2 ng/g and it did not change by the addition of mixtures of amino acids except 'all amino acids $\times 2$' recipe (Figure 4A) ($p>0.05$). Adding all amino acids at twice the level of those naturally present, except asparagine and γ-aminobutyric acid, decreased acrylamide formation by 42% ($p<0.05$). The initial ratio of asparagine to total amino

413 acids was 34% and decreased to 16% when all amino acids (except asparagine and γ-aminobutyric
414 acid) were 3 times their initial level.

415 In a different set of baking, the acrylamide concentration of the control sample was 78±2.2 ng/g
416 (Figure 4B). Addition of 100 mg/kg 2,3-pentanedione and 5% whey protein hydrolysate (both on dry
417 basis) did not change the formation of acrylamide with respect to control ($p>0.05$). On the other
418 hand, a 33% increase in acrylamide was observed when 3,4-hexanedione (100 mg/kg on dry basis)
419 was added alone ($p<0.05$) and this effect was not apparent when it was added together with amino
420 acids. The increase in acrylamide formation by addition of 3,4-hexanedione could stem from the
421 presence of 4-hydroxy-3-hexanone in the aroma as an impurity which was identified in GC-MS
422 chromatogram. It has been shown that α-hydroxy functionality in carbonyl compounds favours
423 acrylamide formation (Stadler, Robert, Riediker, Varga, Davidek, Devaud, et al., 2004).

424 **3.8 *Colour***

425 The addition of free amino acids or α-dicarbonyl compounds did not change surface browning. On
426 the other hand, in samples where whey protein hydrolysate had been added there was more surface
427 browning and sensory analysis was performed under red light.

428 ***3.9 Sensory Analysis of Selected Recipes***

429 Quantitative descriptive sensory evaluation of selected amino acid added samples showed that
430 increasing alkylpyrazines (Gly(×100)+Ala) and Strecker aldehydes (Leu+Ile+Val+Met) separately did
431 not have any effect on baked aroma and flavour (Table 1) ($p>0.05$). Increasing alkylpyrazines and
432 Strecker aldehydes together, as in Gly(×100)+Ala+Leu+Ile+Val+Met, was found to be necessary for
433 an increase in the perception of baked aroma and flavour. This was the case for overall aroma
434 intensity and overall flavour intensity as well. There were no changes for other attributes.

435 Interestingly, no significant increase in boiled potato aroma and flavour was observed even though
436 methional was increased considerably. On the other hand, a significant increase for boiled potato
437 aroma was determined in the samples with methionine baked at lower temperature conditions
438 when there was no difference for baked aroma and flavour according to the quantitative descriptive
439 sensory evaluation (data not shown).

440 As mentioned above, the addition of 2,3-pentanedione alone increased the lowest threshold
441 alkylpyrazine 2-ethyl-3,5-dimethylpyrazine by 121% with respect to the control recipe, however, it
442 had no effect on baked aroma and flavour (Table 2). Baked flavour, baked aroma and baked
443 aftertaste increased when 2,3-pentanedione and the Strecker aldehyde precursor amino acids were
444 added together. 3,4-Hexanedione addition together with Leu+Ile+Val+Met+Phe increased baked
445 aroma but not baked flavour. It should be noted that in this set of baking phenylalanine was also
446 added in Strecker mix of amino acids different from the samples given in the previous sensory
447 analysis. As phenylacetaldehyde has a floral and honey like flavour, it changed the flavour profile
448 and panellist were agreed on the presence of a cheesy attribute in certain samples during the
449 training session of this set of samples and it was added to the vocabulary list. Quantitative
450 descriptive sensory evaluation indicated that cheesy attribute appeared when there is
451 phenylalanine in the recipe. Only the control and the sample containing just 2,3-pentanedione had
452 a score of zero for cheesy. It should be mentioned that no buttery aroma and flavour was described
453 by the panellists in 2,3-pentanedione added samples during training sessions and this was later
454 assessed by introducing butter and also food grade 2,3-pentanedione aroma solution to the
455 panellists. Similarly, panellist did not describe 3,4-hexanedione aroma and flavour (caramel, nutty,
456 buttery) in these samples as confirmed also by introducing its food grade solution to the panellist.

457 In all whey protein hydrolysate added samples, overall aroma intensity was found to be higher but
458 there were no differences for baked aroma and flavour even though there were considerable

459 increases in alkylpyrazines (sensory data not shown). As mentioned above, there were no increases
460 in Strecker aldehydes in these samples. Therefore, increasing only alkylpyrazines did not contribute
461 to the perception of baked aroma and flavour when there were no increments of Strecker
462 aldehydes.

463 **4. Conclusion**

464 This work shows that it is possible to enhance baked flavour in low acrylamide potato-based
465 products (~80 ng/g acrylamide), by adding a specifically targeted combination of amino acids and
466 key intermediates, without increasing acrylamide concentration. Adding the full complement of free
467 amino acids (excluding asparagine) did not have the same effect, nor did addition of hydrolysed
468 whey protein. The best combination was glycine, alanine, valine, leucine, isoleucine and methionine,
469 a combination which was required to boost both Strecker aldehydes and pyrazines. The data add to
470 our understanding of the role of precursor mixtures and highlights merit for a targeted approach
471 over a single source of protein. Appropriate selection of food ingredients to reflect the desired
472 amino acid profile would be required. Similar chemistry has been demonstrated in binary or tertiary
473 aqueous model systems, but one of the achievements of this paper is to show how this can be
474 applied in a complex food matrix where up to 6 amino acids were used to achieve the desired effect.
475

476 **Declaration**

477 This study was funded by PepsiCo. Co-author, Avinash Kant, is employed by PepsiCo. The views
478 expressed in this article are those of the authors and do not necessarily reflect the position or policy
479 of PepsiCo, Inc.

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561 **Figure Captions**

562 **Figure 1.** Relative amounts of Strecker aldehydes and selected alkylpyrazines formed in a potato-
563 based matrix with or without the addition of amino acids. Bars with the same lowercase letters
564 above the bars indicate no statistically significant difference between them ($p>0.05$).

565 **Figure 2.** Effect of 2,3-pentanedione (2,3-PD) and 3,4-hexanedione (3,4-HD) addition on the
566 corresponding alkylpyrazines. Bars with the same lowercase letters above the bars indicate no
567 statistically significant difference between them ($p>0.05$).

568 **Figure 3.** Effect of whey protein hydrolysate (WPH) addition on alkylpyrazine formation also
569 together with 2,3-pentanedione (2,3-PD) and 3,4-hexanedione (3,4-HD). Bars with the same
570 lowercase letters above the bars indicate no statistically significant difference between them
571 ($p>0.05$).

572 **Figure 4.** The concentration of acrylamide formed in different recipes. Samples in A and B graphs
573 are different sets of baking and they have their own control sample. * indicates a statistically
574 significant difference within a group ($p\leq0.05$).

Table 1. Quantitative descriptive sensory evaluation of selected amino acid added samples

	Control	Gly(×100)+Ala	Leu+Ile+ Val+Met	Gly(×100)+Ala+ Leu+Ile+Val+Met	ANOVA p-value
Aroma					
Overall aroma intensity	38^{c}	38^{c}	39^{bc}	44^{a}	0.01
Baked	38^{b}	36^{b}	40^{ab}	43^{a}	0.02
Boiled potato	5	5	6	5	0.74
Fatty	24	23	22	24	0.19
Sweet	13	13	15	16	0.13
Taste					
Sweet	17	16	13	16	0.07
Salty	14	16	15	16	0.26
Bitter	3	1	4	3	0.22
Umami	18	19	17	21	0.24
Flavour					
Overall flavour intensity	38^{c}	39^{c}	40^{bc}	45^{ab}	0.02
Baked	39^{b}	38^{b}	39^{b}	44^{a}	0.01
Boiled potato	6	7	7	7	0.85
Fatty	24	21	20	20	0.08
Aftertaste					
Baked	34	34	34	38	0.07
Boiled potato	4	5	6	5	0.50
Sweet	17	16	15	18	0.15
Salty	15	15	15	16	0.04
Umami	17	17	16	19	0.08
Fatty	18	15	15	16	0.13

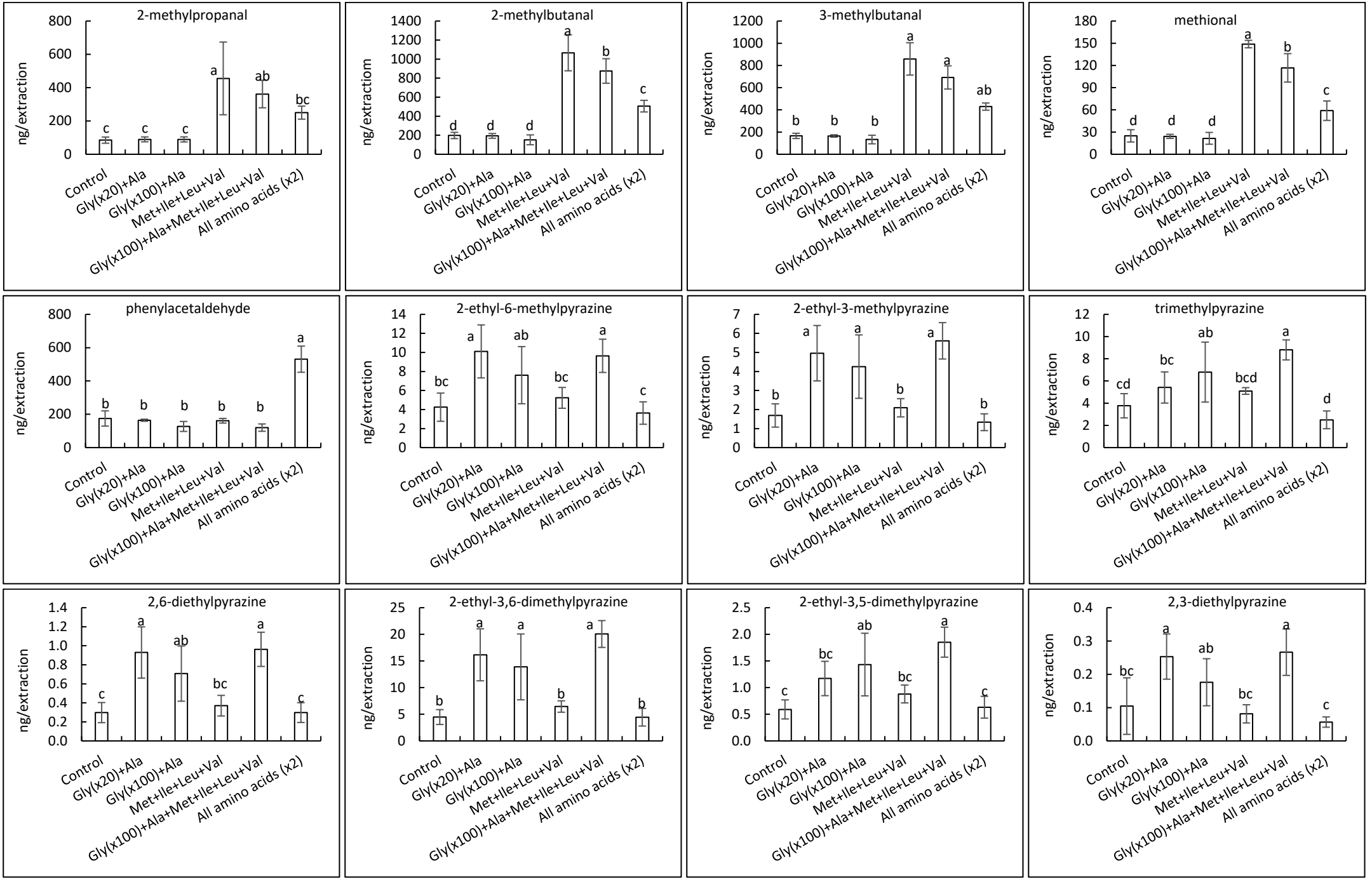
Mean scores (n=12) Within a row, scores with the same lowercase letters are not statistically different ($p>0.05$).

Table 2. Quantitative descriptive sensory evaluation of 2,3-pentanedione and 3,4-hexanedione added samples

	Control	2,3-PD	2,3-PD+Leu+Ile +Val+Met+Phe	3,4-HD+Leu+Ile +Val+Met+Phe	Leu+Ile+Val +Met+Phe	ANOVA	p-value
Aroma							
Overall aroma intensity	33.5^{b}	29.4^{c}	40.3^{a}	36.1^{b}	36.6^{ab}		0.0001
Baked	35.0^{b}	33.2^{b}	42.0^{a}	39.6^{a}	40.4^{a}		0.0015
Boiled potato	5.5	4.7	4.3	4.4	4.3		0.8745
Fatty	17.1^{bc}	15.3^{c}	21.1^{a}	21.3^{a}	19.5^{ab}		0.0011
Sweet	12.1	11.8	12.7	12.4	12.0		0.9731
Cheesy	0.0^{b}	0.0^{b}	5.9^{a}	1.6^{b}	3.6^{ab}		0.0293
Taste							
Sweet	13.2	12.0	12.7	11.8	11.3		0.6764
Salty	13.6	12.5	13.5	13.4	12.8		0.8655
Bitter	2.5^{b}	2.6^{b}	6.8^{a}	6.2^{a}	2.0^{b}		0.0011
Umami	12.4^{bc}	11.1^{c}	15.8^{a}	13.9^{abc}	14.8^{ab}		0.0366
Flavour							
Overall flavour intensity	32.3^{b}	26.7^{c}	43.4^{a}	32.9^{b}	35.2^{b}		<.0001
Baked	32.3^{b}	24.3^{c}	39.7^{a}	33.3^{b}	35.5^{ab}		<.0001
Boiled potato	6.0	7.3	5.1	4.4	3.1		0.2763
Fatty	11.2^{bc}	10.9^{c}	15.9^{a}	14.0^{ab}	14.1^{a}		0.0064
Cheesy	0.0^{b}	0.0^{b}	10.6^{a}	0.8^{b}	4.0^{b}		0.0001
Aftertaste							
Baked	31.0^{b}	23.1^{c}	38.4^{a}	31.7^{b}	33.8^{ab}		0.0003
Boiled potato	5.1^{ab}	5.8^{a}	3.2^{b}	3.9^{ab}	3.1^{b}		0.1390
Sweet	8.6	7.7	9.1	8.0	8.6		0.6608
Salty	11.3	9.3	12.2	10.9	11.5		0.0705
Umami	10.9^{bc}	9.0^{c}	13.9^{a}	11.9^{ab}	12.7^{ab}		0.0127
Fatty	10.4^{bc}	9.2^{c}	13.7^{a}	12.8^{ab}	13.1^{ab}		0.0177
Cheesy	0.0^{b}	0.0^{b}	6.3^{a}	0.5^{b}	2.6^{ab}		0.0261

Mean scores (n=12) Within a row, scores with the same lowercase letters are not statistically different (p>0.05).

Figure 1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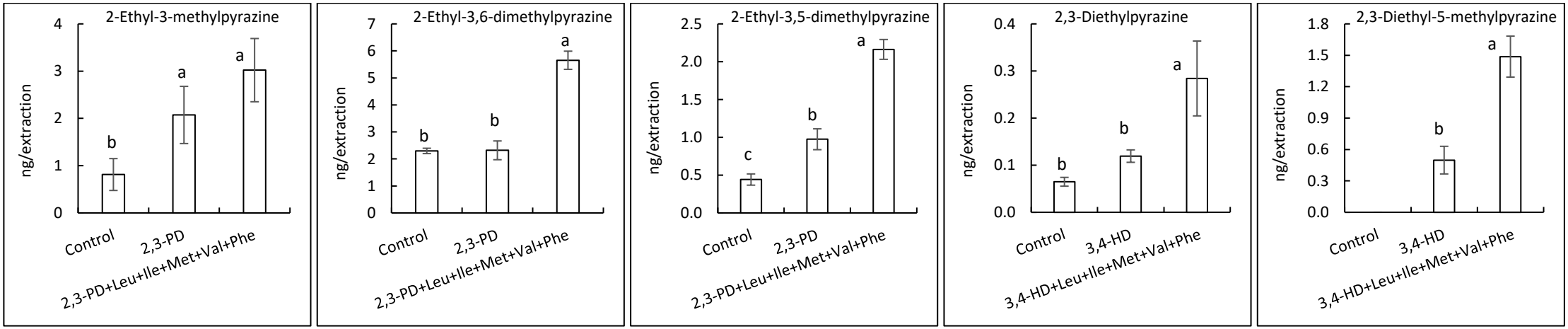
ng/extraction
800
600
400
200
0
c c c a ab bc
2-methylbutanal
ng/extraction
1400
1200
1000
800
600
400
200
0
d d d a b c
3-methylbutanal
ng/extraction
1200
1000
800
600
400
200
0
b b b a a ab
methional
ng/extraction
180
150
120
90
60
30
0
d d d a b c
phenylacetaldehyde
ng/extraction
800
600
400
200
0
b b b b b a
2-ethyl-6-methylpyrazine
ng/extraction
14
12
10
8
6
4
2
0
bc a ab bc a c
2-ethyl-3-methylpyrazine
ng/extraction
7
6
5
4
3
2
1
0
b a a b a b
trimethylpyrazine
ng/extraction
12
10
8
6
4
2
0
cd bc ab bcd a d
2,6-diethylpyrazine
ng/extraction
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
c a ab bc a c
2-ethyl-3,6-dimethylpyrazine
ng/extraction
25
20
15
10
5
0
b a a b a b
2-ethyl-3,5-dimethylpyrazine
ng/extraction
2.5
2.0
1.5
1.0
0.5
0.0
c bc ab bc a c
2,3-diethylpyrazine
ng/extraction
0.4
0.3
0.2
0.1
0.0
bc a ab bc a c
Control
Gly(x20)+Ala
Gly(x100)+Ala
Met+Ile+Leu+Val
Gly(x100)+Ala+Met+Ile+Leu+Val
All amino acids (x2)

Figure 2


2-Ethyl-3-methylpyrazine
ng/extraction
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3
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b
a
a
Control
2,3-PD
2,3-PD+Leu+Ile+Met+Val+Phe
2-Ethyl-3,6-dimethylpyrazine
ng/extraction
0
1
2
3
4
5
6
7
b
b
a
Control
2,3-PD
2,3-PD+Leu+Ile+Met+Val+Phe
2-Ethyl-3,5-dimethylpyrazine
ng/extraction
0.0
0.5
1.0
1.5
2.0
2.5
c
b
a
Control
2,3-PD
2,3-PD+Leu+Ile+Met+Val+Phe
2,3-Diethylpyrazine
ng/extraction
0.0
0.1
0.2
0.3
0.4
b
b
a
Control
3,4-HD
3,4-HD+Leu+Ile+Met+Val+Phe
2,3-Diethyl-5-methylpyrazine
ng/extraction
0.0
0.3
0.6
0.9
1.2
1.5
1.8
b
a
Control
3,4-HD
3,4-HD+Leu+Ile+Met+Val+Phe

Figure 3

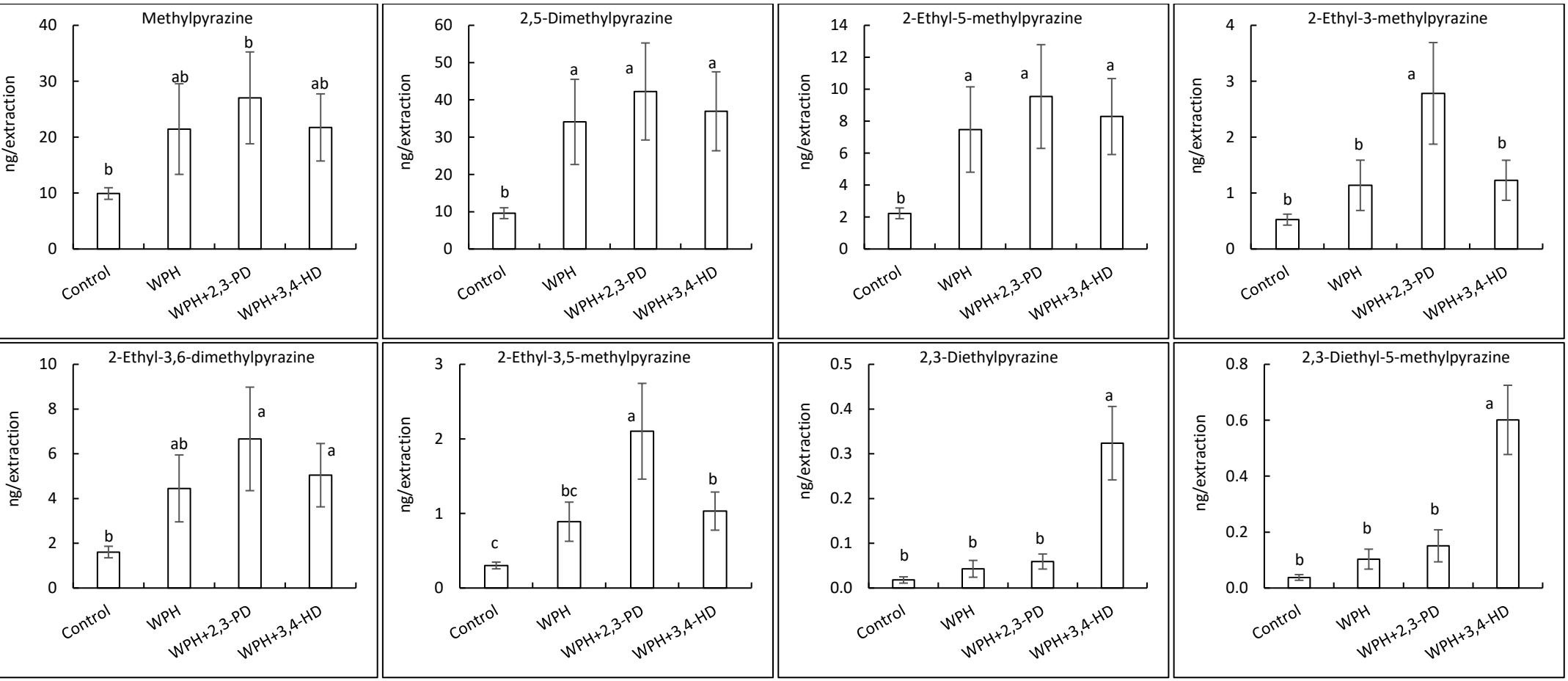

Methylpyrazine
ng/extraction
40
30
20
10
0
b
ab
b
ab
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2,5-Dimethylpyrazine
ng/extraction
60
50
40
30
20
10
0
b
a
a
a
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2-Ethyl-5-methylpyrazine
ng/extraction
14
12
10
8
6
4
2
0
b
a
a
a
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2-Ethyl-3-methylpyrazine
ng/extraction
4
3
2
1
0
b
b
a
b
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2-Ethyl-3,6-dimethylpyrazine
ng/extraction
10
8
6
4
2
0
b
ab
a
a
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2-Ethyl-3,5-methylpyrazine
ng/extraction
3
2
1
0
c
bc
a
b
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2,3-Diethylpyrazine
ng/extraction
0.5
0.4
0.3
0.2
0.1
0.0
b
b
b
a
Control
WPH
WPH+2,3-PD
WPH+3,4-HD
2,3-Diethyl-5-methylpyrazine
ng/extraction
0.8
0.6
0.4
0.2
0.0
b
b
b
a
Control
WPH
WPH+2,3-PD
WPH+3,4-HD

Figure 4

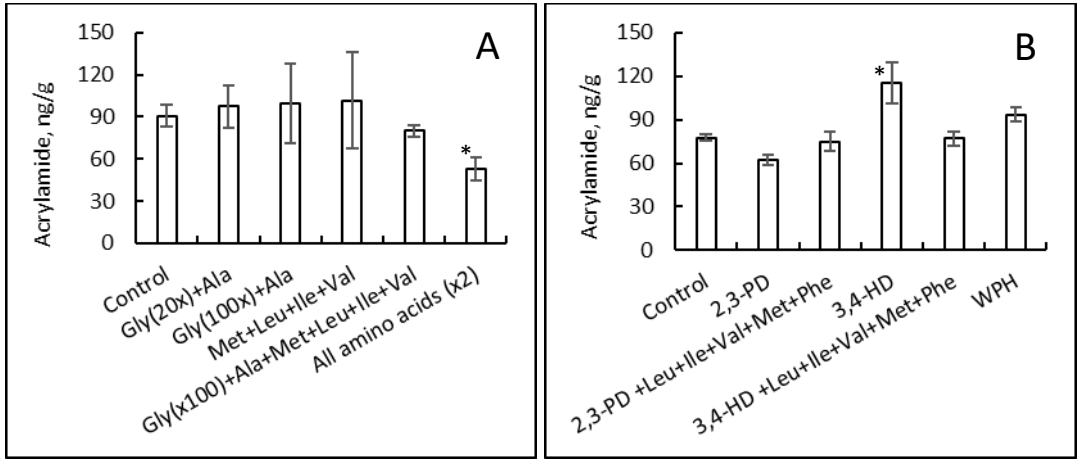

A
Acrylamide, ng/g
150
120
90
60
30
0
*
Control
Gly(20x)+Ala
Gly(100x)+Ala
Met+Leu+Ile+Val
Gly(x100)+Ala+Met+Leu+Ile+Val
All amino acids (x2)
B
Acrylamide, ng/g
150
120
90
60
30
0
*
Control
2,3-PD
2,3-PD +Leu+Ile+Val+Met+Phe
3,4-HD
3,4-HD +Leu+Ile+Val+Met+Phe
WPH

Figure S1. Formation of Strecker aldehydes and selected alkylpyrazines by addition of individual amino acids and also binary mixtures of amino acids during baking at 140 ˚C for 8 min followed by drying at 110 ˚C for 15 min (unless indicated amounts of added amino acids are 4-times the naturally occurring levels)

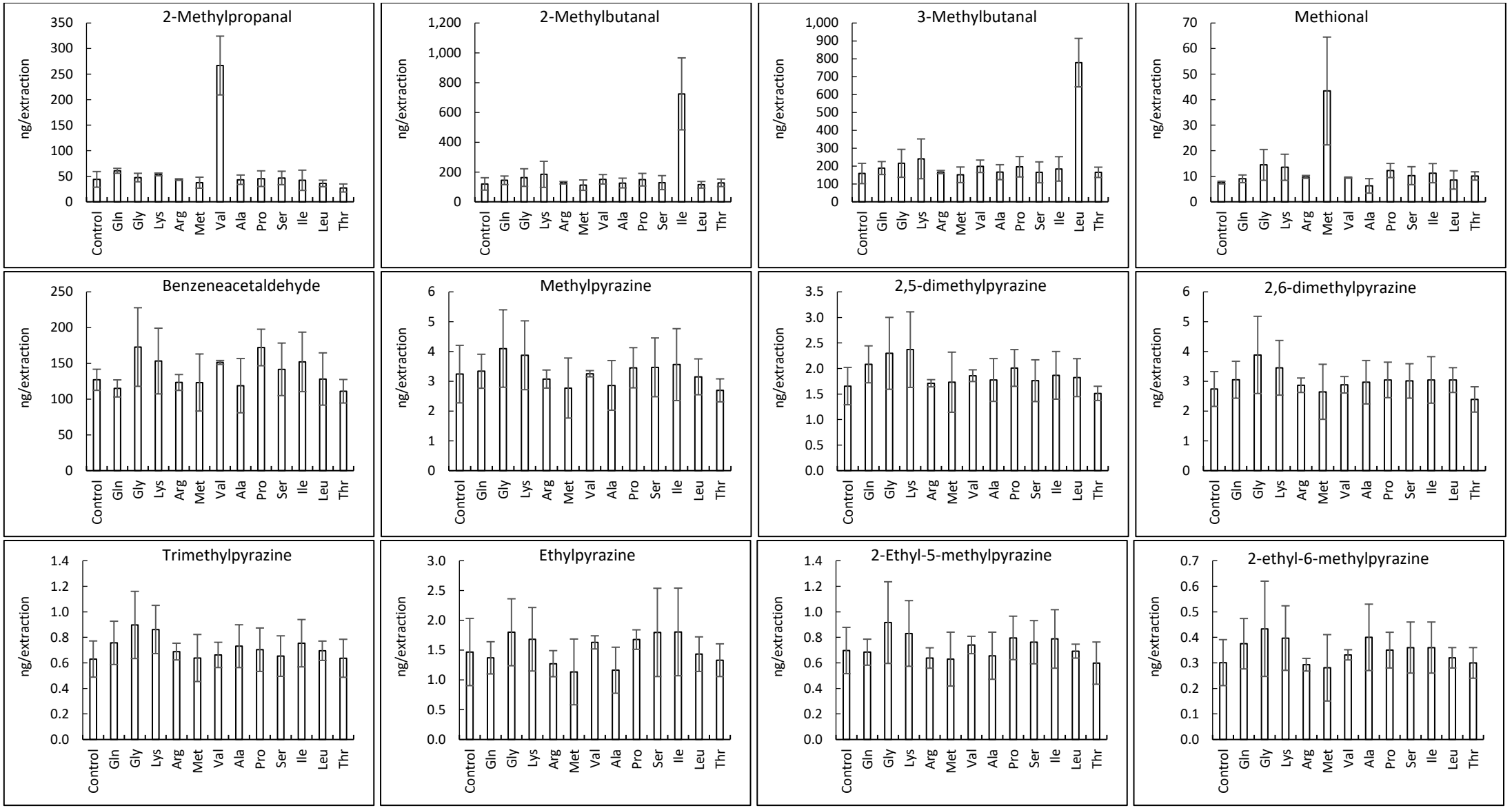


Figure S1 (continued)

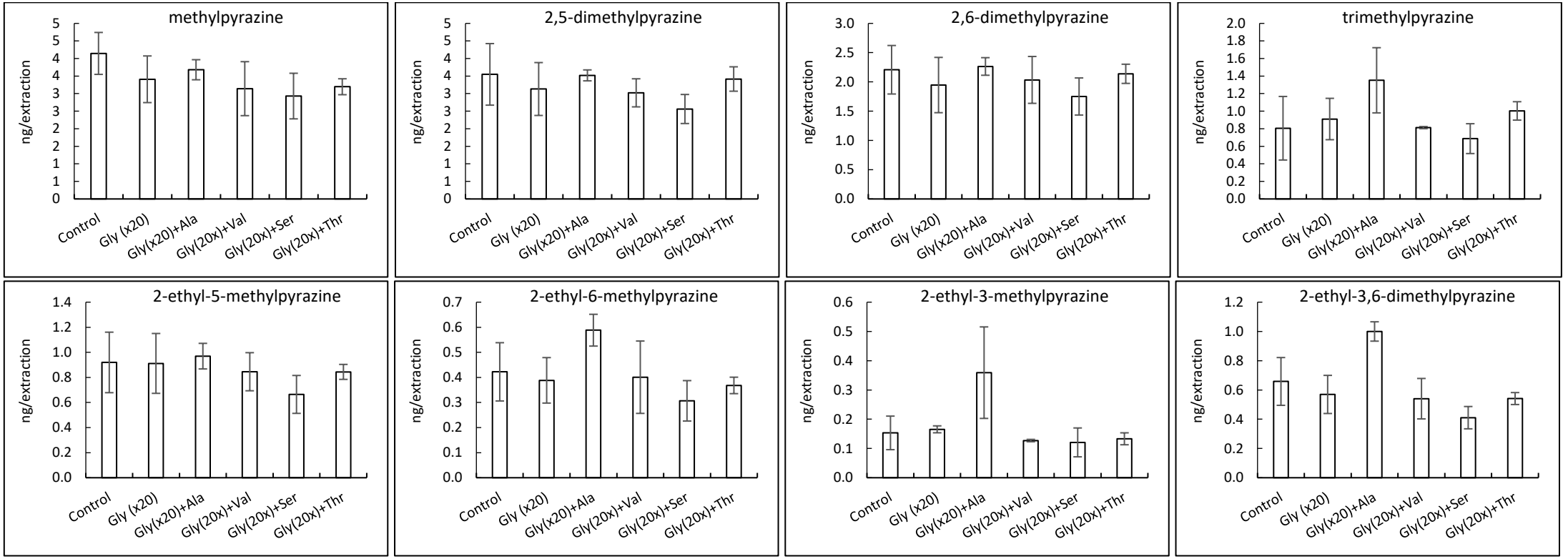


Figure S2. Formation of selected alkylpyrazines by addition of individual amino acids and also binary mixtures of amino acids during baking at 160 ˚C for 6 min followed by drying at 140 ˚C for 8 min (Unless indicated amounts of added amino acids are 4-times the naturally occurring levels)

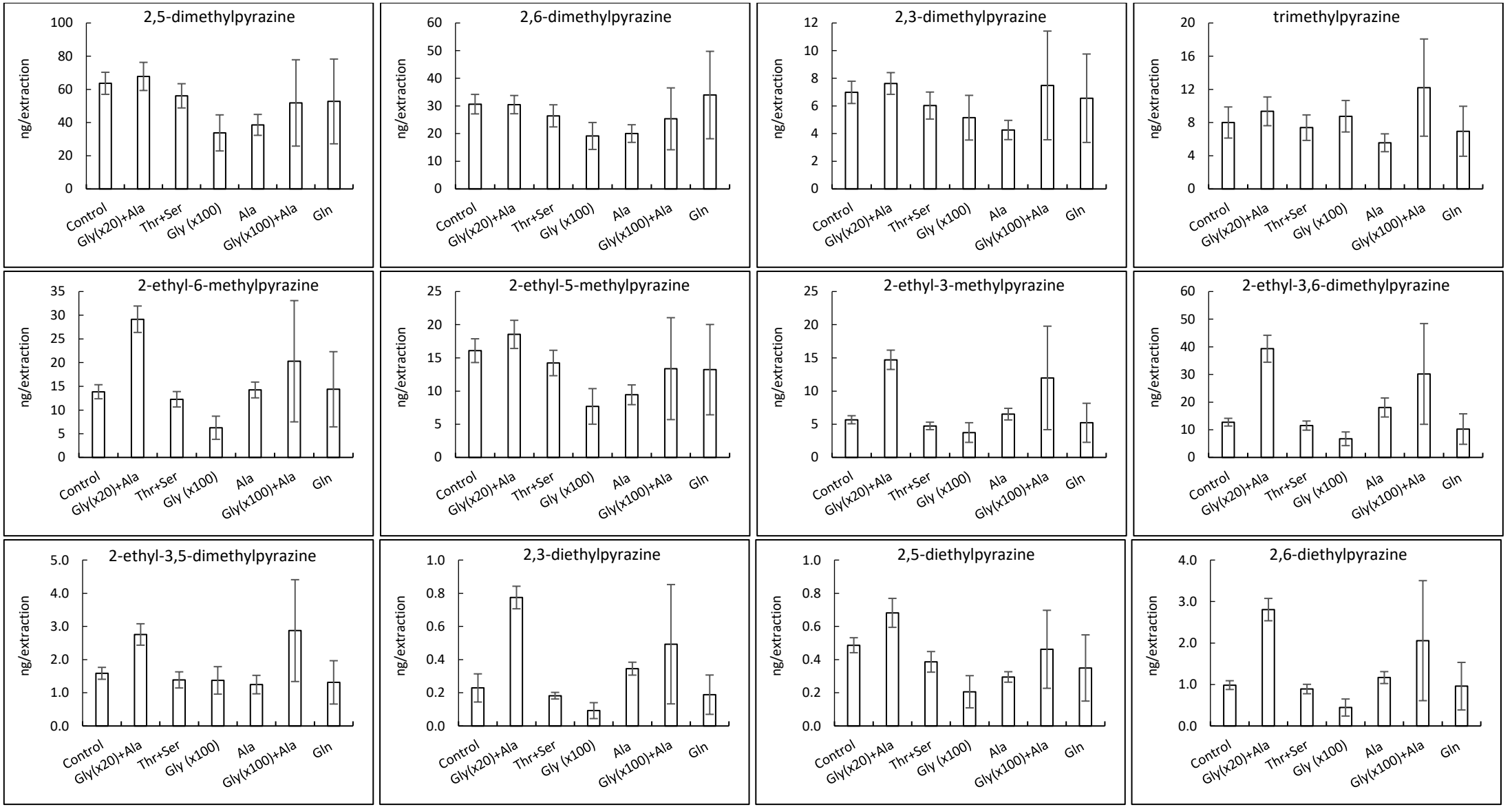
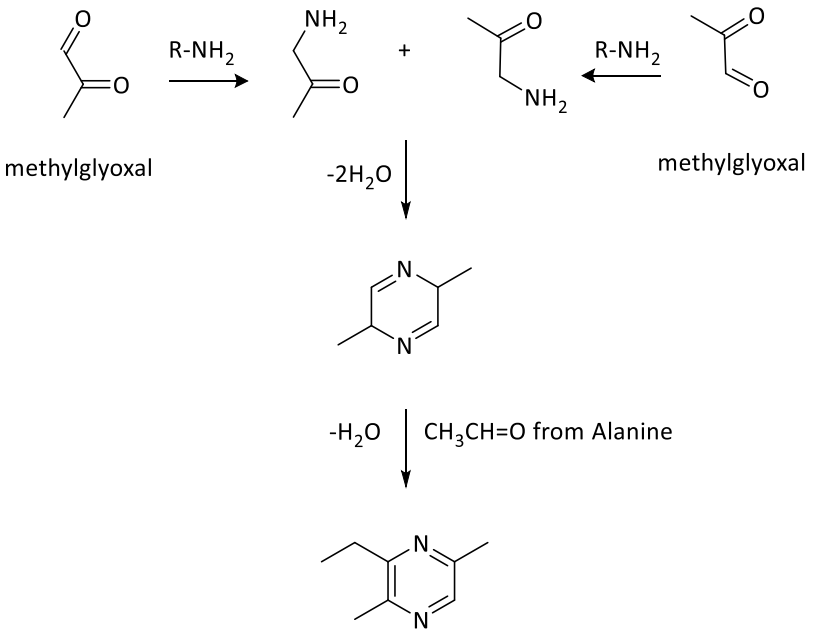
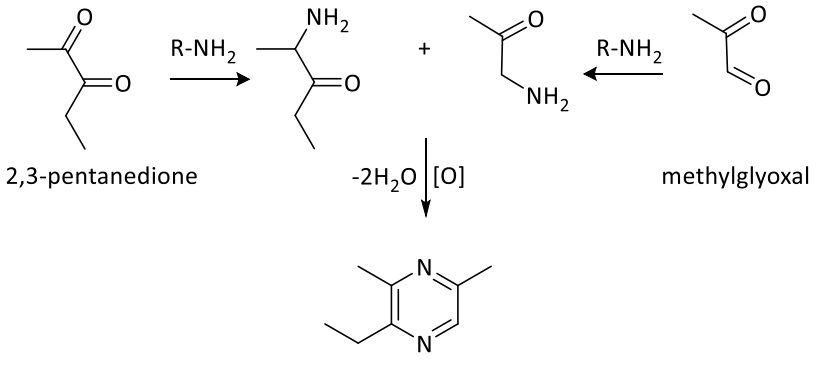


Figure S3. Formation of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine



2-ethyl-3,6-dimethylpyrazine



2-ethyl-3,5-dimethylpyrazine

Table S1. Concentrations of free amino acids (mg/kg) and reducing sugars (g/kg) in potato flake and the final concentration (mg/kg) of added amino acids in the recipes on potato flake basis

	Potao flake	Recipes in Figure 1					Recipes in Figure 2		Recipes in Figure S1 and S2																	
Amino acid	Concentration (mg/kg)	Gly(x20) +Ala	Gly(x100) +Ala	Met +Ile +Leu +Val	Gly(x100) +Ala+Met +Ile+Leu +Val	AA (x2)	2,3-PD	2,3-PD +Met+Ile +Leu+Val +Phe	Gln	Gly	Lys	Arg	Met	Val	Ala	Pro	Ser	Ile	Leu	Thr	Gly (x20)	Gly (x20) +Ala	Gly (x20) +Val	Gly (x20) +Thr	Thr +Ser	Gly (x100)	Gly (x100) +Ala
Ala	531±1.3	2655	2655		2655	1593									2655							2655					2655
Arg	66±18.2					198						330															
Asn	2552±36.4																										
Asp	585±27.5					1755																					
Cys	nd*																										
GABA	580±4.3																										
Gln	401±2.8					2005			2005																		
Glu	952±31.9					2856																					
Gly	18±0.6	378	1818		1818	54				90											378	378	378	378		1818	1818
His	90±0.4					270																					
Ile	157±3.3			785	785	471		785										785									
Leu	168±5.9			840	840	504		840											840								
Lys	35±5.3					105					175																
Met	107±0.2			535	535	321		535					535														
Phe	223±3.1					669		1115																			
Pro	97±1.1					291										485											
Ser	68±0.6					204											340								340		
Thr	98±0.2					294														490				490	490		
Trp	67±2.1					201																					
Tyr	250±2.0					750																					
Val	235±1.6			1175	1175	705		1175						1175									1175				

Sugar	Concentration (g/kg)
Glucose	6.7±0.24
Fructose	3.7±0.04

*nd: not detected

Table S2. Effect of amino acid addition on the formation of volatile Maillard reaction products (ng/extraction) during baking at 160 ˚C for 6 min followed by drying at 130 ˚C for 10 min (unless indicated amounts of added amino acids are 4-times the naturally occurring levels) (Within a row, values with the same lowercase letters are not statistically different at p=0.05.

	r threshold in g/L)ø	ol	!0)+Ala	l00)+Ala	le+Leu+Val	l00)+Ala+ Met+Ile+Leu+Val	ino acids (×2) except Asn
2-Methylpropanal [A]		902	85±19 [c]	89±15 [c]	89±15 [c]	456±218 [a]	362±83 [ab]	250±39 [bc]
2-Methylbutanal [A]		1003	197±33 [d]	193±25 [d]	152±52 [d]	1066±189 [a]	875±129 [b]	506±61 [c]
3-Methylbutanal [A]		1008	166±24 [b]	165±11 [b]	133±39 [b]	859±146 [a]	692±105 [a]	429±33 [ab]
Methional [A]		1465	25±8 [d]	24±3 [d]	22±8 [d]	149±5 [a]	117±19 [b]	59±13 [c]
Phenylacetaldehyde [A]		1634	175±46 [b]	164±6 [b]	127±30 [b]	161±14 [b]	120±22 [b]	531±79 [a]
2,3-Butanedione [A]		1057	37±4.5 [abc]	39±2.2 [ab]	45±10 [a]	34±5.4 [bc]	37±5.9 [abc]	28±2.3 [c]
2,3-Pentanedione [A]		1130	26±4.4 [ab]	31±2.8 [a]	28±8.9 [a]	23±2.9 [ab]	24±4.4 [ab]	17±1.6 [b]
Dimethyl disulfide [A]		1136	44±6.3 [cd]	39±4.2 [cd]	32±7.0 [d]	88±19 [ab]	106±26 [a]	64±9.2 [bc]
Dimethyl trisulfide [A]		1387	5.2±2.4 [b]	4.5±1.1 [b]	6.2±2.5 [b]	7.3±0.3 [b]	13±2.9 [a]	7.4±1.6 [b]
Pyrazine [A]		1258	3.1±1.1 [a]	3.1±0.70 [a]	2.3±0.59 [a]	3.5±0.76 [a]	2.7±0.33 [a]	2.4±0.52 [a]
Methylpyrazine [A]	>2000	1296	24±8.0 [ab]	27±6.3 [a]	20±7.1 [ab]	29±6.1 [a]	23±3.5 [ab]	14±3.9 [b]
2,5-Dimethylpyrazine [A]	1820	1343	21±6.7 [ab]	30±8.5 [a]	25±11 [ab]	30±3.6 [a]	33±3.7 [a]	13±4.7 [b]
2,6-Dimethylpyrazine [A]	1720	1348	12±3.5 [ab]	15±3.3 [a]	13±5.2 [ab]	14±0.66 [ab]	15±2.4 [a]	7.9±2.1 [b]
Ethylpyrazine [A]	>2000	1351	13±4.6 [ab]	14±3.0 [ab]	9±3.1 [ab]	16±4.2 [a]	11±2.3 [ab]	8.9±2.3 [b]
2,3-Dimethylpyrazine [A]	880	1363	2.5±0.81 [bc]	3.3±0.79 [ab]	3.4±1.3 [ab]	3.2±0.40 [ab]	4.0±0.58 [a]	1.6±0.48 [c]
2-Ethyl-6-methylpyrazine [A]		1393	4.2±1.5 [bc]	10±2.8 [a]	7.6±3.0 [ab]	5.2±1.1 [bc]	9.6±1.7 [a]	3.6±1.2 [c]
2-Ethyl-5-methylpyrazine [A]		1398	5.6±1.8 [ab]	7.8±2.1[a]	6.2±2.6 [ab]	7.9±1.5 [a]	8.6±1.1 [a]	4.1±1.4 [b]
2-Ethyl-3-methylpyrazine [A]	35	1411	1.7±0.61 [b]	5.0±1.5 [a]	4.3±1.7 [a]	2.1±0.48 [b]	5.6±1.0 [a]	1.3±0.44 [b]
Trimethylpyrazine [A]	50	1412	3.8±1.1 [cd]	5.4±1.4 [bc]	6.8±2.7 [ab]	5.1±0.30 [bcd]	8.8±0.90 [a]	2.5±0.80 [d]
2,6-Diethylpyrazine [A]	1.7	1437	0.30±0.11 [c]	0.93±0.27 [a]	0.71±0.29 [ab]	0.37±0.11 [bc]	0.96±0.18 [a]	0.30±0.10 [c]
2-Ethenylpyrazine [B]	>2000	1444	2.0±0.66 [ab]	2.0±0.34 [ab]	1.3±0.44 [b]	2.3±0.55 [a]	1.6±0.32 [ab]	1.2±0.32 [b]
2-Ethyl-3,6-dimethylpyrazine [A]	3.6	1448	4.5±1.4 [b]	16±4.9 [a]	14±6.2 [a]	6.5±1.1 [b]	20±2.5 [a]	4.4±1.7 [b]
2-Ethyl-3,5-dimethylpyrazine [A]	0.011	1464	0.59±0.18 [c]	1.2±0.32 [bc]	1.4±0.59 [ab]	0.88±0.17 [bc]	1.9±0.28 [a]	0.63±0.20 [c]
2,3-Diethylpyrazine [A]	6.6	1456	0.10±0.08 [bc]	0.25±0.07 [a]	0.18±0.07 [ab]	0.08±0.03 [bc]	0.27±0.07 [a]	0.06±0.02 [c]
2,5-Diethylpyrazine [A]	74	1462	0.17±0.05 [bc]	0.28±0.09 [ab]	0.18±0.08 [abc]	0.24±0.06 [abc]	0.30±0.07 [a]	0.13±0.04 [c]
2-Ethenyl-6-methylpyrazine [B]		1492	7.0±2.1 [ab]	9.2±1.6 [a]	7.6±2.8 [ab]	8.3±0.9 [ab]	8.3±1.2 [ab]	5.3±1.5 [b]
2-Ethenyl-5-methylpyrazine [B]		1499	1.6±0.47 [ab]	1.9±0.42 [a]	1.5±0.58 [ab]	2.1±0.41 [a]	1.9±0.26 [a]	1.0±0.27 [b]
2-Ethenyl-3,5(6)-dimethylpyrazine [B]	0.012(870)	1532	1.4±0.39 [bc]	2.0±0.44 [ab]	1.6±0.70 [abc]	1.9±0.34 [ab]	2.4±0.41 [a]	0.94±0.23 [c]
Isopropenylpyrazine [B]		1604	0.21±0.06 [a]	0.21±0.04 [a]	0.21±0.04 [a]	0.21±0.02 [a]	0.24±0.02 [a]	0.22±0.02 [a]
2-furfural [A]		1472	39±8.5 [a]	45±3.9 [a]	42±10.6 [a]	42±1.7 [a]	43±5.1 [a]	40±8.4 [a]

* LRI: Linear retention indices on Phenomenex ZB-Wax column (30 m × 250 µm i.d.× 1 µm), ø Values were taken from Wagner, R., Czerny, M., Bielohradsky, J., & Grosch, W. (1999). Structure odour-activity relationships of alkylpyrazines. Z. Lebensm. Unters. Forsch., 208(5-6), 308-316.

A : Identification was confirmed by LRI (authentic compounds) and mass spectra (NIST library)

B : Identification was confirmed by LRI (literature) and mass spectra (NIST library)

Table S3. Effect of whey protein hydrolysate (WPH), also together with 2,3-pentanedione and 3,4-hexanedione, on the formation of volatile Maillard reaction products (ng/extraction)

	LRI*	Control	WPH	WPH+2,3-PD	WPH+3,4-HD
2-Methylpropanal	902	77±11 ^{a}	87±6.2 ^{a}	108±17 ^{a}	81±36 ^{a}
2-Methylbutanal	1003	145±3.51 ^{a}	161±35.3 ^{a}	176±17 ^{a}	167±24 ^{a}
3-Methylbutanal	1008	144±3.84 ^{a}	137±28.6 ^{a}	145±12 ^{a}	138±18 ^{a}
Methional	1465	11±2.0 ^{a}	11±2.8 ^{a}	13±2.4 ^{a}	12±1.5 ^{a}
Phenylacetaldehyde	1634	88±2.0 ^{a}	77±14 ^{a}	90±12 ^{a}	88±10 ^{a}
2,3-Butanedione	1057	32±3.1 ^{a}	35±4.6 ^{a}	41±1.8 ^{a}	37±7.8 ^{a}
2,3-Pentanedione	1130	13±0.34 ^{b}	21±6.9 ^{b}	260±32 ^{a}	26±5 ^{b}
Dimethyl disulfide	1136	17±1.29 ^{b}	57±2.82 ^{a}	66±11 ^{a}	57±12 ^{a}
Dimethyl trisulfide	1387	1.9±0.39 ^{b}	4.2±0.47 ^{ab}	5.8±2.4 ^{a}	5±0.37 ^{a}
Pyrazine	1258	1.8±0.16 ^{a}	2.3±0.53 ^{a}	2.6±0.41 ^{a}	2±0.45 ^{a}
Methylpyrazine	1296	10±1.0 ^{b}	21±8.1 ^{ab}	27±8.2 ^{b}	22±6.0 ^{ab}
2,5-Dimethylpyrazine	1343	10±1.5 ^{b}	34±11 ^{a}	42±13 ^{a}	37±11 ^{a}
2,6-Dimethylpyrazine	1348	6.5±1.1 ^{b}	10±3.1 ^{ab}	12±2.9 ^{a}	11±2.6 ^{ab}
Ethylpyrazine	1351	4.3±0.72 ^{b}	7.7±3.3 ^{ab}	10±3.4 ^{a}	8±2.4 ^{ab}
2,3-Dimethylpyrazine	1363	1.1±0.13 ^{b}	1.8±0.60 ^{ab}	2.3±0.69 ^{a}	1.9±0.52 ^{ab}
2-Ethyl-6-methylpyrazine	1393	1.5±0.24 ^{b}	3.0±1.1 ^{ab}	4.0±1.4 ^{a}	3.2±0.94 ^{ab}
2-Ethyl-5-methylpyrazine	1398	2.2±0.34 ^{b}	7.5±2.7 ^{a}	9.5±3.2 ^{a}	8.3±2.4 ^{a}
2-Ethyl-3-methylpyrazine	1411	0.53±0.10 ^{b}	1.1±0.45 ^{b}	2.8±0.91 ^{a}	1.2±0.36 ^{b}
Trimethylpyrazine	1412	1.9±0.32 ^{b}	4.9±1.3 ^{a}	5.8±1.6 ^{a}	5.6±1.3 ^{a}
2,6-Diethylpyrazine	1437	0.10±0.02 ^{b}	0.19±0.07 ^{ab}	0.28±0.10 ^{a}	0.22±0.05 ^{ab}
2-Ethenylpyrazine	1444	0.70±0.09 ^{a}	1.1±0.41 ^{a}	1.4±0.42 ^{a}	1.1±0.32 ^{a}
2-Ethyl-3,6-dimethylpyrazine	1448	1.6±0.26 ^{b}	4.5±1.5 ^{ab}	6.7±2.3 ^{a}	5.0±1.4 ^{a}
2-Ethyl-3,5-dimethylpyrazine	1464	0.30±0.05 ^{c}	0.89±0.26 ^{bc}	2.1±0.64 ^{a}	1.0±0.26 ^{b}
2,3-Diethylpyrazine	1456	0.02±0.01 ^{b}	0.04±0.02 ^{b}	0.06±0.02 ^{b}	0.32±0.08 ^{a}
2,5-Diethylpyrazine	1462	0.06±0.00 ^{b}	0.19±0.07 ^{ab}	0.31±0.11 ^{a}	0.21±0.06 ^{a}
2,3-Diethyl-5-methylpyrazine	1485	0.04±0.01 ^{b}	0.10±0.04 ^{b}	0.15±0.06 ^{b}	0.60±0.12 ^{a}
2-Ethenyl-6-methylpyrazine	1492	3.4±0.54 ^{b}	6.2±1.7 ^{ab}	7.4±1.7 ^{a}	6.7±1.7 ^{a}
2-Ethenyl-5-methylpyrazine	1499	0.60±0.09 ^{b}	1.5±0.57 ^{a}	1.9±0.64 ^{a}	1.6±0.45 ^{a}
2-Ethenyl-3,5(6)-dimethylpyrazine	1532	0.60±0.06 ^{b}	1.8±0.56 ^{a}	2.2±0.69 ^{a}	1.9±0.46 ^{a}
2-furfural	1472	30±5.0 ^{a}	28±4.0 ^{a}	29±1.7 ^{a}	29±4.3 ^{a}

Within a row, values with the same lowercase letters are not statistically different at p=0.05.

* LRI: Linear retention indices on Phenomenex ZB-Wax column (30 m × 250 µm i.d.× 1 µm)

Table S4. Sensory characteristics of the samples defined by panellist and their reference materials

Attribute	Reference Material
Aroma	
Overall aroma intensity	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Fatty	Cooked oil
Sweet	
Cheesy	Mild cheddar
Taste	
Sweet	
Salty	
Bitter	
Umami	
Flavour	
Overall flavour intensity	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Fatty	Cooked oil
Cheesy	Mild cheddar
Aftertaste	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Sweet	
Salty	
Umami	
Fatty	Cooked oil
Cheesy	Mild cheddar